

CHAPTER

7

Mycology

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## I. INTRODUCTION AND GENERAL CHARACTERISTICS

### A. Mycology Terms

1. **Moulds:** Multicellular fungi
2. **Yeasts:** Single-cell fungi
3. **Mycosis:** Fungal infection
4. **Systemic mycosis:** Multiorgan infection caused by fungi
5. **Opportunistic mycosis:** Fungal disease that occur primarily in immunocompromised patients
6. **Dimorphic fungi:** Fungi that show both a nonmould (e.g., yeast) and mould phase
7. **Saprobe:** Organism capable of living on decaying organic material

### B. Fungal Structure

1. **Hyphae** are long, branching filaments that come together to form the **mycelium**. There are two main types of hyphae.
  - a. **Septate hyphae** have cellular separation or cross-walls. Septate hyphae range in diameter from 3 to 6  $\mu\text{m}$ .
  - b. **Sparsely septate (formerly aseptate) hyphae** contain few if any cellular separations. Sparsely septate hyphae range in diameter from 5 to 15  $\mu\text{m}$ . **Coenocytic** also refers to hyphae lacking cross-walls.
  - c. **Pseudohyphae** are a chain of cells formed by budding that resemble true hyphae. Pseudohyphae differ from true hyphae in that they are constricted at the septa, form branches that begin with septation, and have terminal cells smaller than other cells.
2. Hyphae are classified as **vegetative** or **aerial**.
  - a. **Vegetative hyphae** function in food absorption and are the portion that extends below the agar surface or nutrient substrate.
  - b. **Aerial hyphae** extend above the agar or nutrient substrate, and their function is to support reproductive structures called **conidia**.
3. Conidia are sporelike asexual reproductive structures not produced by cleavage, conjugation, or free-cell formation. Conidia are only formed by the **imperfect fungi**.
  - a. Conidia morphology is important in fungal identification.
  - b. Conidia classification is based on conidia morphologic development.
  - c. **Microconidia** are single-celled, small conidia.
  - d. **Macroconidia** are multicellular, large conidia.
4. Types of conidia
  - a. **Arthroconidia** are conidia resulting from the fragmentation of hyphae into individual cells. Some fungi will have arthroconidia separated by normal (**disjunct**) cells.
  - b. **Blastoconidia:** Conidia that form as the result of budding
  - c. **Chlamydoconidia** result from terminal cells in the hyphae that enlarge and have thick walls. These conidia can survive adverse environmental

conditions. Chlamydoconidia are found in moulds, whereas similar structures (**chlamydospores**) are found in hyphae produced by some yeasts.

- d. **Poroconidia:** Conidia formed by being pushed through a small pore in the parent cell
- e. **Phialoconidia:** Tube-shaped conidia that can be branched
- f. **Anneloconidia** are vase-shaped conidia; the remaining parent outer cell wall takes on a saw-toothed appearance as the conidia are released.

### C. Sexual and Asexual Reproduction

#### 1. Sexual reproduction

- a. Requires the formation of specialized fungal structures called **spores**
- b. Fungi that undergo sexual reproduction are termed **perfect fungi**.
- c. Types of spores
  - 1) **Ascospores:** Spores contained in a saclike structure
  - 2) **Basidiospores:** Spores contained in a club-shaped structure
  - 3) **Oospores:** Spores resulting from the fusion of cells from two different hyphae
  - 4) **Zygospores:** Spores resulting from the fusion of two identical hyphae

#### 2. Asexual reproduction

- a. Asexual reproduction only involves division of the nucleus and cytoplasm.
- b. Fungi that undergo asexual reproduction are termed **imperfect fungi**.
- c. **Imperfect fungi are the only fungal group to produce conidia.**

## II. CULTURE AND ISOLATION

### A. Types of Fungal Media

#### 1. Sabouraud dextrose agar (SDA)

- a. General-purpose, nutritionally poor medium mildly selective for fungi, no longer commonly used; several different formulations available
- b. In one formulation, the agar has an acidic pH (5.6) that inhibits most bacteria. Modified SDA (Emmons) has a neutral pH and better supports the growth of fungi but is less inhibitory for bacteria.

#### 2. Sabouraud-brain heart infusion agar (SABHI)

- a. A nonselective medium for isolation of all fungi
- b. Contains dextrose, peptone, and brain heart infusion
- c. Can be made selective for dimorphic fungi by the addition of **cyclohexamide, chloramphenicol, and gentamicin**

#### 3. Brain heart infusion agar with blood (BHIB)

- a. Used to grow most fungi, especially those from sterile body sites
- b. Contains brain heart infusion and sheep blood
- c. Can be made selective for dimorphic fungi by the addition of **cyclohexamide, chloramphenicol, and gentamicin**. Cyclohexamide

inhibits the saprophytic fungi and chloramphenicol inhibits many gram-positive and gram-negative bacteria, whereas gentamicin inhibits primarily gram-negative bacteria.

4. **Selective agars** contain various antimicrobial agents that will enhance the growth of specific fungal pathogens and will inhibit bacteria and other undesired growth.
  - a. **Inhibitory mould agar (IMA)**
    - 1) IMA is used to grow most fungal pathogens; it is especially formulated to recover the cyclohexamide-sensitive *Cryptococcus*.
    - 2) Contains gentamicin and chloramphenicol
  - b. **Dermatophyte test medium (DTM)**
    - 1) Used to isolate the dermatophytes
    - 2) DTM contains cyclohexamide and gentamicin and phenol red as a pH indicator.
5. **Differential agars** are used to enhance pigment development, conidia production, and mould-to-yeast phase transition.
  - a. **Potato dextrose agar (PDA)**
    - 1) Used to enhance conidia development
    - 2) Enhances pigment development of *Trichophyton rubrum*
  - b. **Bird seed (niger seed) and caffeic acid agars** are selective and differential media used to grow *C. neoformans*. *C. neoformans* forms black to brown colonies due to the activity of **phenol oxidase**. Chloramphenicol can be added to make the media selective.
  - c. **Cornmeal agar with Tween 80:** Used to differentiate *Candida* spp.
  - d. **Agars containing rice, casein, and other nutrients** are used to differentiate *Trichophyton* spp.

## B. Culture Considerations

1. **Fungal cultures are incubated at 30°C.**
2. Growth requires from several days to several weeks.
3. Cultures should be maintained in a high-humidity environment.
4. Several techniques are used to obtain culture material for slide preparation.
  - a. **Tease mount method:** A dissecting needle is used to pull apart a fungal colony, which is placed on a slide. This method may damage fungal structure, especially conidia. It may take several attempts to obtain a specimen with intact conidia.
  - b. **Cellophane tape method:** Cellophane tape is used to transfer aerial hyphae from the colony to a microscope slide for examination.
  - c. The **slide culture method** uses a block of agar overlaid with a cover slip. Fungal colonies are grown on the side of the agar block. The cover slip is removed and used for microscopic examination. This method prevents damage to the fungal structure.

### C. Direct Examination Methods

1. **Saline wet mount** is used to view fungal elements, such as hyphae, conidia, and budding yeasts. It has limited use and is most commonly applicable for vaginal secretions to diagnose vaginitis.
2. **Lactophenol cotton blue wet mount** is used to stain and preserve fungal elements in culture isolates.
3. **Potassium hydroxide (KOH)** is used to dissolve nonfungal materials in skin, nail, and hair samples.
4. **Gram stain** can be used to view yeasts.
5. **India ink** can be used to reveal capsules surrounding *C. neoformans* found in cerebrospinal fluid (CSF). However, due to low sensitivity, direct antigen detection assays have generally replaced the India ink wet mount.
6. **Calcofluor white stain** is a fluorochrome that stains chitin found in the cell wall of fungi. The stain is not absorbed by human tissue. The slide is viewed using an ultraviolet light. Fungi will appear white to blue to green depending on the wavelength of light. KOH can be added to clear the specimen of cellular debris.

### III. BODY SITES AND POSSIBLE FUNGAL PATHOGENS

- A. **Blood:** *Candida* spp., *Blastomyces dermatitidis*, *Histoplasma capsulatum*, and *Cryptococcus neoformans*
- B. **Cerebrospinal Fluid:** *Cryptococcus neoformans*, *Candida* spp., *Histoplasma capsulatum*, and *Coccidioides immitis*
- C. **Hair:** *Microsporum* and *Trichophyton*
- D. **Nails:** *Aspergillus*, *Epidermophyton*, and *Trichophyton*
- E. **Skin:** *Candida*, *Microsporum*, *Trichophyton*, *Epidermophyton*, and *Blastomyces dermatitidis*
- F. **Lungs:** *Candida albicans*, *Aspergillus*, *Rhizopus*, *Penicillium*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis*
- G. **Throat:** *Candida albicans* and *Geotrichum candidum*
- H. **Urine:** *Candida albicans* and *Candida glabrata*
- I. **Genital Tract:** *Candida albicans*

### IV. YEASTS

#### A. Introduction

1. Yeasts are common causes of vaginitis and urinary tract infections (UTIs) in women and can cause a number of other diseases in healthy and immunosuppressed individuals. In addition, yeast can cause newborn infections and meningitis. The most common cause of yeast infections is *Candida albicans*.

## 2. Methods for identification

### a. Microscopic appearance

- 1) Saline wet mounts and Gram stains will show budding yeast.
- 2) Yeasts are discovered in routine urinalysis.
- 3) **India ink** preparations are used to show the capsule surrounding *Cryptococcus neoformans*.

### b. Culturing

- 1) Yeasts are grown on SABHI at 22–30°C.
- 2) Yeasts will form cream-colored, mucoid to smooth colonies within several days. On blood agar, yeast colonies can resemble *Staphylococcus* colonies.
- 3) **Cornmeal agar with Tween 80** is used to differentiate *Candida* spp. by enhancing the formation of fungal elements such as hyphae, pseudohyphae, and conidia.
- 4) *C. albicans* will show **chlamydospores** with clusters of blastoconidia along the hyphae.
- 5) *C. tropicalis* typically produces long-branched pseudohyphae. Blastoconidia are produced singly or in short chains. This species does not produce chlamydospores.

### c. Germ tube production

- 1) **Germ tubes** are hyphaelike extensions of young yeast cells showing parallel sides, are nonseptate (showing no cell wall division), and will not constrict at their point of origin. **Pseudohyphae** look like germ tubes but are septate and constricted at their point of origin.
- 2) Germ tube procedure: Yeasts are incubated with serum at 37°C for up to 3 hours and examined for germ tube production.
- 3) *C. albicans* is positive for germ tube production. *Candida tropicalis* is used for the negative control; however, some strains can produce germ tubes if incubated over 3 hours.

### d. Carbohydrate assimilation test

- 1) Assimilation tests determine the aerobic utilization of carbohydrates.
- 2) Agar slants containing various carbohydrates are inoculated with yeast suspended in saline. The medium contains the pH indicator bromocresol purple. The tubes are incubated at room temperature and read at 7 and 14 days. Use of the carbohydrates results in the formation of yellow colonies.
- 3) A number of commercially prepared tests based on carbohydrate utilization and enzyme hydrolysis are also available.

### e. Urease test

- 1) Used to identify *Cryptococcus* spp., which are urease **positive**.
- 2) *C. albicans* is used for the negative control.
- 3) A positive urease is indicated by a pink to purple color.

- f. **Chromagars** allow for the identification of several species of yeasts. The media contain a variety of substrates. The ability to metabolize different substrates results in the production of colonies of different colors.

## B. Clinically Significant Yeasts

### 1. *Candida albicans*

- a. *C. albicans* is the most common yeast isolate and is the causative agent of candidiasis, a general term for *Candida* infections.
- b. *C. albicans* is normal flora of the mucous membranes lining the respiratory, gastrointestinal, and female genital tracts. Most adult infections are **endogenous**, whereas infants acquire infections from their mothers (**exogenous** infections).
- c. **Types of candidiasis**
  - 1) Thrush (oral cavity)
  - 2) Vulvovaginitis (vagina)
  - 3) Onychomycosis (nail infections)
  - 4) Paronychomycosis (cuticle infections)
- d. *C. albicans* can also cause systemic infections, including meningitis, UTIs, and heart and lung infections.
- e. Predisposition to *Candida* infections includes burns, wounds, diabetes mellitus, antimicrobial therapy, pregnancy, leukemia, and immune problems.
- f. **Culture characteristics**
  - 1) *C. albicans* grows on most fungal media as well as sheep blood, chocolate, and eosin-methylene blue agars.
  - 2) On cornmeal agar with Tween 80, isolates produce chlamydospores.
  - 3) **Biochemical tests**
    - a) A positive germ tube can be a presumptive identification of *C. albicans*; however, not all strains are positive. *C. dubliniensis* is also positive and will form chlamydospores.
    - b) Except for *C. krusei*, all *Candida* are urease negative. Not all strains of *C. krusei* are urease positive.
    - c) *Candida* spp. are inositol negative.
- g. **Other clinically important species:** *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, etc.

### 2. *Cryptococcus neoformans*

- a. Causes cryptococcosis, which can produce a mild to moderate pulmonary infection; however, in the immunocompromised patient, cryptococcosis can lead to systemic infections and meningitis. Cryptococcosis is also associated with prostate and tissue infections.
- b. *C. neoformans* can be acquired by contact with bat, pigeon, or other bird droppings, in addition to contaminated vegetables, fruit, and milk.



- c. **Identifying characteristics for direct specimens**
    - 1) On Gram stain the yeasts appear in spherical form and are not of uniform size.
    - 2) Hematoxylin and eosin stains are used to show capsules in tissue.
    - 3) Direct antigen test for cryptococcal antigen: Performed on CSF and serum specimens
  - d. **Culture characteristics**
    - 1) Brown to black colonies on bird seed or caffeic acid agars
    - 2) Only forms blastoconidia
    - 3) **Biochemical tests**
      - a) Positive for urease and phenol oxidase
      - b) Inositol utilization
      - c) Negative for nitrate reduction
3. ***Trichosporon***
- a. *T. beigelii* was the name formerly used for the species in the genus *Trichosporon* causing most human infections. Recent taxonomic changes question the validity of *T. beigelii* as a species name. Several other species are now associated with human infections, including the human hair infection **white piedra** and rarely the systemic disease referred to as trichosporonosis.
  - b. *Trichosporon* spp. can be isolated from the soil, animals, and humans.
  - c. **Culture characteristics**
    - 1) *Trichosporon* spp. form cream-colored, smooth colonies on solid media in about 1 week.
    - 2) Hyaline hyphae with blastoconidia and arthroconidia are produced.
    - 3) **Biochemical tests**
      - a) Positive for urease
      - b) Can assimilate some carbohydrates
4. ***Rhodotorula***
- a. *Rhodotorula* spp. are found in moist environments such as on shower curtains and toothbrushes. They have also been isolated from soil and dairy products. Although they have been associated with hospital-acquired infections, they are generally considered commensals or contaminants.
  - b. *Rhodotorula* resemble the *Cryptococcus*, but they are inositol negative. Some species produce a pink pigment.
5. ***Geotrichum candidum*** is actually a mould that can be confused with yeast based on colony morphology. Microscopically, *G. candidum* forms true hyphae with rectangular **arthroconidia**. This fungus has been isolated from a number of clinical specimens, but its clinical significance is questionable.



## V. OPPORTUNISTIC FUNGI

### A. Introduction

1. Many fungi rarely cause disease in healthy individuals, but they can cause disease in individuals with medical conditions (e.g., diabetes) and in immunosuppressed patients.
2. General characteristics of opportunistic fungi
  - a. Most opportunistic fungi form colonies within several days (rapid growers).
  - b. Humans generally acquire infections through inhalation of the conidia.
  - c. Most opportunistic fungi live on organic matter (**saprophytic fungi**) found in the soil.
  - d. **Laboratory identification**
    - 1) Opportunistic fungi are inhibited by many antimicrobial agents (e.g., cyclohexamide); therefore, media should not contain these substances when trying to isolate opportunistic fungi.
    - 2) Because they are frequent contaminants and are found in high numbers in the environment, opportunistic fungi **must be repeatedly isolated in patients** to be considered significant.
    - 3) Identification is based on microscopic morphology. The hyphae are **hyaline** (lightly pigmented).

### B. Clinically Significant Opportunistic Fungi

1. *Aspergillus* spp.
  - a. Causes **aspergillosis**, which can affect the skin, heart, lungs, and central nervous system. Pulmonary aspergillosis affects the bronchi, lungs, or sinuses.
  - b. *Aspergillus fumigatus* is the most common cause of aspergillosis. *A. niger* is an important cause of **otomycosis**, a superficial mycotic infection of the outer ear canal characterized by inflammation, pruritus, and scaling.
  - c. **Identifying characteristics**
    - 1) **Colony morphology:** *Aspergillus* spp. form granular/fluffy or powdery growth within 2 days on SABHI. Pigmentation varies according to species.
    - 2) **Microscopic appearance:** Hyphae are septate; **conidiophores** terminate in a large, spherical vesicle bearing **phialides**.
  - d. **Species identification** is based on colony appearance and microscopic characteristics. *Aspergillus* spp. have **septate hyaline hyphae**. Conidiophores arise from a **foot cell** and support a single **vesicle** at their tip. Flask-shaped phialides, in a single or double row, produce chains of **phialoconidia**.
    - 1) *A. niger* colonies are yellow to black with a yellow reverse.

- 2) *A. flavus* colonies are green to brown with red-brown reverse.
  - 3) *A. terreus* colonies are green to yellow with yellow reverse.
  - 4) *A. clavatus* colonies are blue to green with white reverse.
  - 5) *A. fumigatus* colonies are green to gray with tan reverse.
2. **Zygomycetes**
- a. Members of the class Zygomycetes include *Absidia*, *Mucor*, *Rhizomucor*, *Rhizopus*, and *Syncephalastrum*.
  - b. Cause of infections is known as **zygomycoses** and **mucormycoses**.
    - 1) Produce allergic reactions in susceptible individuals
    - 2) Mucormycoses are uncommon in otherwise healthy individuals. Infections of the paranasal sinuses that can extend to the central nervous system (rhinocerebral) are probably the most common. Infections can rapidly progress to a fatal outcome in immunocompromised patients and in diabetics with ketoacidosis.
    - 3) Spores gain entry (e.g., via inhalation) into body sites and can cause infections in those areas.
    - 4) Some zygomycetes produce toxins that can cause gastrointestinal disturbances.
    - 5) Blood infections (**fungemia**) can lead to central nervous system disorders.
  - c. **Identifying characteristics**
    - 1) **Colony morphology:** Growth after several days is dense; colonies show a cotton candy texture, and pigmentation ranges from white, to gray, to brown.
    - 2) **Microscopic appearance:** Hyaline hyphae are sparsely septate and are ribbonlike and thin walled.
    - 3) Zygomycetes typically form **rhizoids**, which resemble tree roots and function in attachment and nutrient absorption.
  - d. **Species identification**
    - 1) *Absidia* spp. exhibit branching **sporangiophores** between the **rhizoid** (rootlike hyphae). A slight swelling below the **columella** at the base of the **sporangia** is present.
    - 2) *Mucor* spp.: Single or branching sporangiophores are present, but rhizoids are absent. No swelling is noted below the columella.
    - 3) *Rhizopus* spp. produce unbranched sporangiophores that arise opposite rhizoids. No swelling is noted below the columella.
3. **Fusarium**
- a. *Fusarium* spp. are opportunistic fungi associated with a variety of clinical presentations, including mycetomas, keratitis, and systemic infections.
  - b. **Identifying characteristics**
    - 1) **Colony morphology:** Initially *Fusarium* produces white, cottony colonies that quickly develop pink or violet centers.

- 2) **Microscopic appearance:** They form septate hyphae and two forms of conidiation: (1) conidiophores, with phialides producing large, sickle-shaped macroconidia with 3–5 septa; and (2) simple conidiophores, with small, oval conidia singularly or in clusters.

## VI. CUTANEOUS AND SUPERFICIAL FUNGI

### A. Introduction

1. **Superficial mycoses** are infections that involve the outer epithelial layers of the skin and top layers of the hair and nails.
2. **Cutaneous mycoses** involve deeper layers of the skin and more tissue.
3. **Dermatophyte** is the term used to group the various fungi that cause infections (dermatophytoses) of the skin, hair, and nails.
  - a. The dermatophytes are **keratinophilic** (i.e., able to metabolize keratin).
  - b. Dermatophytes contain three genera.
    - 1) ***Trichophyton***: Infects nails, hair, and skin
    - 2) ***Epidermophyton***: Infects skin and nails
    - 3) ***Microsporum***: Infects hair and skin
4. Superficial and cutaneous fungi are rarely invasive to other areas of the body.
5. Dermatophyte skin infection is termed **tinea**.
6. **Types of tinea infections and their causative agents**
  - a. **Tinea pedis** or **athlete's foot**: An infection of the spaces between the toes
    - 1) Caused by *Trichophyton* spp. and *Epidermophyton* spp.
    - 2) Characterized by itching and scaling
  - b. **Tinea corporis** or **ringworm**: An infection of smooth skin
    - 1) Caused by *Microsporum* spp. and *Trichophyton* spp.
    - 2) Characterized by circular patches of scaly skin
  - c. **Tinea unguium** or **onychomycosis**: An infection of the nails
    - 1) Caused by *Epidermophyton* spp. and *Trichophyton* spp.
    - 2) Characterized by discoloration, thickening, and progressive destruction of the nails
  - d. **Tinea capitis**: An infection of the scalp
    - 1) Caused by *Microsporum* spp. and *Trichophyton* spp.
    - 2) Characterized by circular bald patches on the scalp
  - e. **Tinea barbae** or **barber's itch**: An infection of beard hair
    - 1) Caused by *Microsporum* spp. and *Trichophyton* spp.
    - 2) Characterized by skin lesions
  - f. **Tinea cruris** or **jock itch**: An infection of the groin
    - 1) Caused by *Trichophyton* spp. and *Epidermophyton* spp.
    - 2) Characterized by itching and scaling of the groin area
7. Identification of the dermatophytes is primarily based on colony morphology and microscopic appearance. In some cases, it may be necessary to perform an *in vitro* **hair perforation test**. Sterile hair is infected with the isolated fungus and after incubation is examined microscopically for wedge-shaped perforations.

## B. Characteristics of the Dermatophytes

### 1. *Trichophyton*

- a. **Colony characteristics:** Two colony types will be seen between 7 and 10 days on SABHI at room temperature.
  - 1) **Buff granular colonies**, rose to tan colored, with a yellow, brown, or red reverse
  - 2) **White fluffy colonies** with a colorless to yellow reverse
- b. **Microscopic characteristics**
  - 1) **Macroconidia** are smooth/thin walled, pencil shaped, contain 3–7 cells, and are few in number. See Figure 7-1 ■.
  - 2) **Microconidia** are round to club shaped in grapelike clusters and are few to numerous in number.

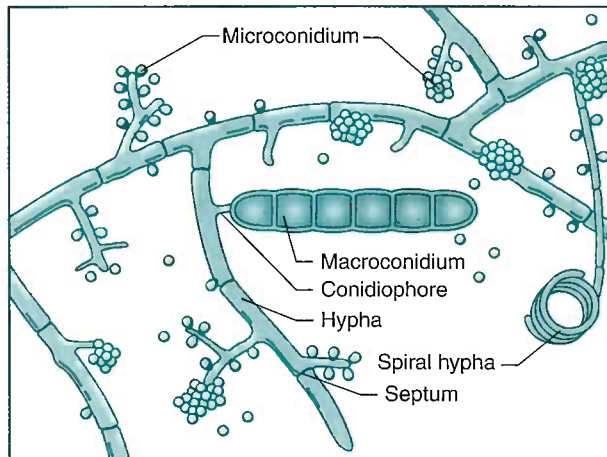


FIGURE 7-1 ■ *Trichophyton*

- c. **Species identification**
  - 1) *T. mentagrophytes* characteristically produce grapelike clusters of microconidia. Some strains produce numerous macroconidia. *T. mentagrophytes* is positive in the hair perforation test.
  - 2) *T. rubrum* produces few or numerous macroconidia; numerous club-shaped microconidia are borne singularly on the hyphae. *T. rubrum* forms deep red reverse on PDA. *T. rubrum* is negative in the hair perforation test.
  - 3) *T. verrucosum* produces only chlamydoconidia on SDA or PDA. On thiamine-enriched media, **elongated rat-tail macroconidia** are produced. *T. verrucosum* is negative in the hair perforation test.
2. *Epidermophyton*
  - a. **Culture characteristics:** On SABHI at room temperature, colonies will appear yellow with a tan reverse within 10 days.
  - b. **Microscopic characteristics**

- 1) **Macroconidia** are smooth/thin walled, club shaped, contain 2–5 cells, and are numerous in number. See Figure 7-2■.

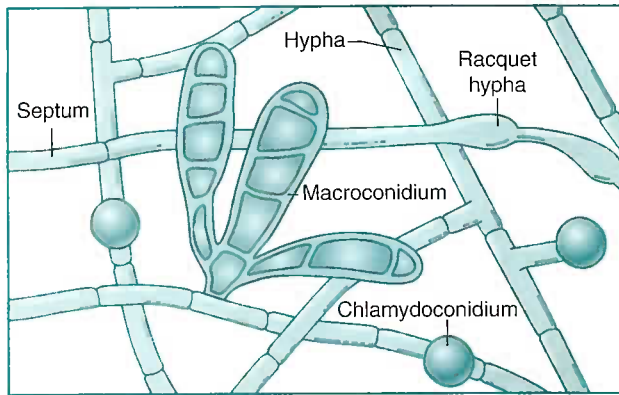


FIGURE 7-2 ■ *Epidermophyton*

- 2) **Microconidia** are not present.
  - 3) *E. floccosum* invades nails, and on KOH preparation chains of arthroconidia can be seen.
- c. At room temperature on SDA, *E. floccosum* forms khaki-yellow colonies with tan reverse.
3. ***Microsporum***
- a. **Colony morphology:** On SABHI at room temperature, colonies will be light tan, with a salmon-colored reverse. *Microsporum* spp. are very slow growers.
  - b. **Microscopic characteristics**
    - 1) **Macroconidia** are rough/thin to thick walled, spindle shaped, contain 4–15 cells, and are numerous in number. See Figure 7-3■.
    - 2) **Microconidia** are club shaped, single, and few in number.

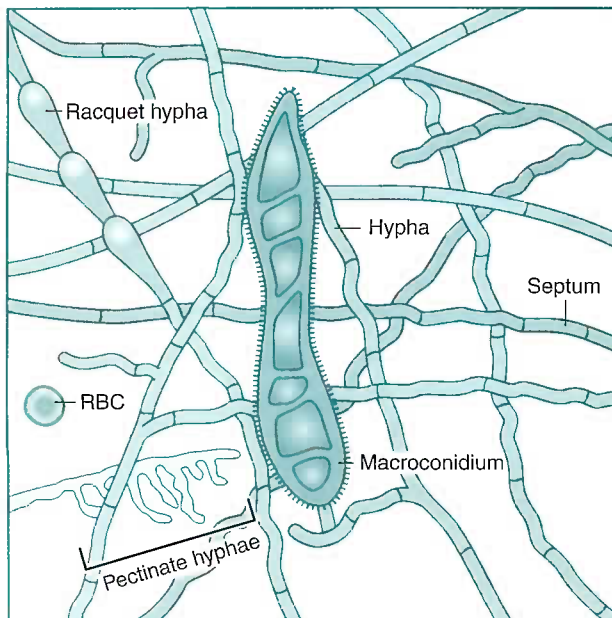


FIGURE 7-3 ■ *Microsporum*

## c. Species identification

- 1) *M. audouinii* forms pectinate (comblike) septate hyphae with terminal chlamydoconidia often with pointed ends. Unlike other dermatophytes, this species grows poorly on rice grains. It is an **anthropophilic** (found in humans) species.
- 2) *M. canis* forms numerous thick-walled, spindle-shaped macroconidia with tapered ends and 6–15 cells. It is a **zoophilic** species (found in animals).
- 3) *M. gypseum* produces numerous thin-walled, elliptical macroconidia containing 4–6 cells. It is a **geophilic** species (found in the soil).

## C. Superficial Mycoses

1. **Tinea (pityriasis) versicolor**

- a. Infection of the stratum corneum caused by lipophilic yeast belonging to the *Malassezia furfur* complex. Infection commonly occurs on the upper back, chest, shoulders, upper arms, and abdomen. There may be an association between the disease and excessive sweating.
- b. Diagnosis is made by KOH preparation of skin scrapings from the lesions that demonstrate characteristic yeastlike cells and hyphae (spaghetti and meatballs). Most lesions will fluoresce yellow under a Wood's lamp.

2. **Tinea nigra**

- a. Tinea nigra is characterized by the presence of brown to black nonscaly macules on the palms of the hands and less commonly the dorsa of the feet. Infections are most commonly caused by *Hortaea werneckii*; synonyms are *Phaeoannellomyces werneckii*, *Exophiala werneckii*, and *Cladosporium werneckii*.
- b. The presence of numerous light brown, frequently branching septate hyphae and budding cells (some with septates) on KOH preparations is suggestive of infection.

3. **Black piedra**

- a. Black piedra is a fungal infection of the scalp hair and less frequently the beard, mustache, and axillary and pubic hairs. The disease is characterized by the presence of hard, dark nodules on the hair shaft. *Piedra hortaea* is the causative agent.
- b. Diagnosis can be made by submerging hair in a solution of 25% KOH or NaOH with 5% glycerol and heating. Microscopic examination will reveal compact masses of dark, septate hyphae and round to oval **asci** containing 2–8 hyaline, aseptate banana-shaped **ascospores**.

4. **White piedra**

- a. White piedra is a fungal infection of facial, axillary, or genital hairs and less commonly the scalp. It is characterized by the presence of soft, white, yellowish, beige, or greenish nodules on the hair shaft. *Trichosporon ovoides* is the causative agent of scalp infections, whereas *T. inkin* causes most cases of pubic white piedra.



- b. Microscopic evaluation of hair treated in 10% KOH or 25% NaOH with 5% glycerol reveals intertwined hyaline septate hyphae breaking up into oval or rectangular **arthroconidia**. Culture characteristics of the *Trichosporon* spp. were previously discussed with the yeasts.

## VII. SUBCUTANEOUS FUNGI

### A. Introduction

1. Fungi causing subcutaneous mycoses can gain entry into the subcutaneous tissue via trauma to the skin.
2. Resulting subcutaneous lesions are characterized by being chronic, hard, crusted, and ulcerated.
3. Humans acquire the infections from vegetation contaminated with the fungi. The feet are commonly affected.
4. Subcutaneous mycoses are mainly caused by **dematiaceous fungi**, which is a group of slowly growing fungi found in the soil and vegetation. “Dematiaceous” refers to hyphae that appear darkly pigmented, olive green, brown, and black when viewed microscopically.

### B. Subcutaneous Mycoses

1. **Mycetoma** is a granulomatous infection of the subcutaneous tissue causing cutaneous abscesses. Exudate from mycetomas will contain red, yellow, or black granules. Most infections are found in Africa. Causative agents include *Pseudoallescheria boydii*, *Exophiala*, *Acremonium*, and *Madurella*.
2. **Chromoblastomycosis** is a localized infection characterized by chronic, hard, or tumorlike lesions. Most infections involve the feet or lower legs. It is seen mostly in tropical areas of the world. Most infections are caused by *Fonsecaea pedrosoi*. Other fungi causing chromoblastomycosis are *Phialophora*, *Cladosporium*, *Exophiala*, and *Wangiella*. The presence of **sclerotic bodies** (copper-colored fungal cells) in lesions is characteristic. Colonies are folded or heaped and are gray to black.
3. **Phaeohyphomycosis** is a superficial or subcutaneous infection that can become systemic. Resulting systemic infections can cause endocarditis and brain abscesses. Fungi causing phaeohyphomycosis include *Bipolaris*, *Curvularia*, and *Phialophora*. *Alternaria*, a dematiaceous fungus generally considered a saprophyte, has been associated with some cutaneous infections.
4. **Sporotrichosis** is a subcutaneous infection; lymph and pulmonary infections can also occur. Sporotrichosis is known as rose gardener’s disease. Infections can come from rose thorns and contact with sphagnum moss. *Sporothrix schenckii*, the cause of sporotrichosis, is a **dimorphic** fungus. When grown on media with blood at 35°C, these fungi grow as small yeasts. When grown on SDA or PDA at room temperature, they are in the mould phase characterized by delicate hyphae and microconidia. Yeast cells may be seen in segmented neutrophils and are “cigar shaped.”



## VIII. SYSTEMIC FUNGI

### A. Introduction

1. This fungal group is often acquired via inhalation and can disseminate to any of the body's organ systems.
2. Most systemic fungi are dimorphic, exhibiting a nonmould (e.g., yeast) parasitic phase at 35–37°C and a mould (or mycelial) saprobic phase at 25–30°C.
3. **Identifying characteristics**
  - a. Identification is based on temperature and medium requirements and colony and microscopic morphology.
  - b. Most systemic dimorphic fungi are very slow growers and require 3–7 weeks to grow.
  - c. Because the **mould forms are highly infective**, slants are used for culture.
  - d. Colonies are membranous and develop tan aerial mycelia.
  - e. Conidia identification is necessary in species identification.
  - f. **Conversion of dimorphic fungi from the mould to yeast phase is confirmation that the fungus in question is dimorphic.**
4. **Systemic dimorphic fungi**
  - a. *Blastomyces dermatitidis* (blastomycosis)
  - b. *Coccidioides immitis* (coccidioidomycosis)
  - c. *Histoplasma capsulatum* (histoplasmosis)
  - d. *Paracoccidioides brasiliensis* (paracoccidioidomycosis)

### B. Description of the Agents

1. ***Blastomyces dermatitidis***
  - a. Blastomycosis is a respiratory infection that can affect the skin and bones. Infections are acquired by inhalation of conidia or hyphae and can be mild to chronic.
  - b. The precise environmental location of this fungus is unknown. Outbreaks have occurred following contact with moist environments such as streams and rivers and contact with decaying vegetation. Cases in the U.S. occur most frequently in the Ohio and Mississippi River basins. More cases occur in males than in females.
  - c. *B. dermatitidis* can be cultured from tissue or body fluids.
  - d. **Identifying characteristics**
    - 1) **Microscopic appearance**
      - a) The **mould phase** is characterized by the presence of single smooth-walled, round to oval conidia at the ends of short conidiophores. The mould phase of *B. dermatitidis* can be confused with *Scedosporium apiospermum* or *Chrysosporium* spp. *S. apiospermum* is the causative agent of **mycetoma** and can infect the brain, bones, eyes, lungs, etc. *Chrysosporium* is commonly considered a contaminant.

- b) **Yeast phase:** Large, round, thick-walled, budding yeasts with broad-based blastoconidia

## 2) Culture

- a) At room temperature, initially a yeastlike colony develops, and over time the colony will become fluffy white to tan.
- b) Conversion from the mould to yeast phase requires 4–6 days.

## 2. *Coccidioides immitis*

- a. Coccidioidomycosis (valley fever) is an infection of the lungs, bones, joints, skin, lymph nodes, central nervous system, and adrenal glands. Infections can be acute or chronic and self-limiting or requiring medications.
- b. Most infections in the U.S. are in the semiarid southwest desert region (Lower Sonoran Life Zone). Infections are sometimes called desert or valley fever in the San Joaquin Valley of California, where many cases are diagnosed.
- c. Infections are often acquired through spore inhalation from the environment. Activities that increase airborne dust, such as plowing and construction, can facilitate transmission.

## d. Identifying characteristics

### 1) Microscopic appearance

- a) Branching thick-walled, rectangular (barrel-shaped) **arthroconidia**
- b) Tissue phase shows round, thick-walled **spherule** filled with small **endospores**. The tissue phase can only be grown under special conditions *in vitro*.

### 2) Culture

- a) At 37°C on SABHI agar, colonies will appear moist and white and turn fluffy white in about a week.
- b) **As with all mould phase fungi, always use a biological safety cabinet to prevent inhalation of spores.**

## 3. *Histoplasma capsulatum*

- a. Histoplasmosis can be a fatal pulmonary infection but can also affect the spleen, liver, kidneys, bone marrow, and heart.
- b. Infection is acquired by spore inhalation from barns, chicken houses, and bat caves. *H. capsulatum* has been associated with **guano**, in particular from starlings and bats.
- c. Most infections occur in the southern and Midwestern U.S. and along the Appalachian Mountains. The major risk factor for infection is environmental exposure.

## d. Identifying characteristics

### 1) Microscopic appearance

- a) The mould phase will show conidiophores at 90-degree angles to hyphae supporting smooth macroconidia (8–16 µm in diameter) with finlike edges (**tuberculate**). Microconidia are small (2–5 µm in diameter) and round to teardrop shaped.

- b) Yeasts appear as **small** single-budding cells that are unremarkable in morphology. In clinical specimens, yeasts are often found inside monocytes and macrophages.
- 2) **Culture**
  - a) On blood-containing media, the colonies are initially moist and develop tan aerial mycelia.
  - b) Mature colonies are woolly and velvety and appear tan colored.
- 4. *Paracoccidioides brasiliensis*
  - a. Paracoccidioidomycosis is a chronic granulomatous disease of the lungs and skin that can spread to the liver and spleen.
  - b. Mostly found in South America
  - c. Acquired by spore inhalation or ingestion
  - d. **Identifying characteristics**
    - 1) **Microscopic appearance**
      - a) Yeast cells grown at 35–37°C are thick walled, with multiple budding yeast cells with very narrow necks.
      - b) The mould phase exhibits mostly hyphae with intercalary and terminal chlamydoconidia.
    - 2) **Culture**
      - a) When grown on blood-containing media at 35–37°C, the colonies are waxy, wrinkled, and cream to tan colored.
      - b) When grown on SDA or PDA at room temperature, colonies are initially smooth. Colonies become tan with aerial mycelium.
- 5. *Penicillium marneffe*
  - a. *P. marneffe* is unique among the members of the genus *Penicillium* in that it is dimorphic and a true pathogen.
  - b. **Identifying characteristics**
    - 1) The **yeast cells** are oval and small (3–8  $\mu\text{m}$ ) and resemble *H. capsulatum*.
    - 2) At 22–30°C, structures typical of the genus *Penicillium* develop. Green aerial mycelium and reddish-brown hyphae are produced along with a red diffusible pigment.
- 6. *Pneumocystis*
  - a. *Pneumocystis* spp. are nonfilamentous (do not produce hyphae) fungi found in the **lower respiratory tract** of humans and other animals.
  - b. Four species of *Pneumocystis* have been named; *P. jirovecii* is the name currently given to the species that infects humans.
  - c. In healthy individuals, infections are generally asymptomatic. However, in immunocompromised patients, such as those with **acquired immunodeficiency syndrome** (AIDS), the infection can result in a serious or fatal pneumonia. *Pneumocystis* pneumonia remains an important opportunistic infection in patients with AIDS.

d. **Diagnosis**

- 1) *P. jirovecii* are found primarily in the **lungs**. Specimens used for the detection of this fungus include bronchoalveolar lavage, induced sputum, open lung biopsy, transbronchial aspirate, and nasopharyngeal aspirates.
- 2) Nucleic acid probes and amplification assays
- 3) **Microscopic examination**
  - a) **Stains:** Methanamine silver, periodic acid-Schiff, Giemsa, calcofluor white, etc.
  - b) **Microscopic appearance:** Cysts (8  $\mu\text{m}$ ) contain several intracystic bodies, trophozoites (2–3  $\mu\text{m}$ ) with dark staining nuclei (depending on the stain).



## review

## questions

**INSTRUCTIONS** Each of the questions or incomplete statements that follows is comprised of four suggested responses. Select the *best* answer or completion statement in each case.

1. A bulldozer operator became ill while working on a new highway in the San Joaquin Valley. He developed chest pain, anorexia, headache and general malaise, and myalgia with fever. Chest X-ray showed pneumonic infiltrate and a single, well-defined nodule in the left lower lobe. His leukocyte count and sedimentation rate were slightly elevated. Although no fungus was seen in direct examination of a sputum specimen, processing included a culture on Sabouraud dextrose agar with chloramphenicol and cycloheximide. Within 3 days at 30°C, this culture produced moist, grayish growth, and white aerial mycelia began to develop (see Color Plate 30■). A lactophenol cotton blue wet mount of this organism is seen in Color Plate 31■. What is the most likely identification of this fungus?
  - A. *Aspergillus fumigatus*
  - B. *Blastomyces dermatitidis*
  - C. *Coccidioides immitis*
  - D. *Histoplasma capsulatum*
2. A 38-year-old male from Ohio presented to his physician with a mild influenzalike illness that included headache and malaise. His chest X-ray showed no infiltrates. His past medical history was unremarkable. He had no history of travel but reported recently cleaning the bell tower at his church, which was littered with bird excrement. The most likely agent causing his disease is
  - A. *Aspergillus fumigatus*
  - B. *Coccidioides immitis*
  - C. *Candida albicans*
  - D. *Histoplasma capsulatum*

3. A 44-year-old gardener pricked herself with a rose thorn. A subcutaneous fungal infection characterized by the development of necrotic ulcers followed this direct inoculation of fungal spores into the skin. The causative fungus was cultured as a small yeast form at 35°C (see Color Plate 32 ■) and as a mould at room temperature with delicate hyphae and conidia. This disease is
  - A. Blastomycosis
  - B. Chromomycosis
  - C. Mycetoma
  - D. Sporotrichosis
4. A yeastlike fungus was isolated from a sputum sample. No hyphae were produced on cornmeal agar with Tween 80. The isolate was negative for nitrate assimilation and positive for inositol assimilation and produced urease at 37°C. These findings are typical of
  - A. *Candida krusei*
  - B. *Cryptococcus terreus*
  - C. *Cryptococcus neoformans*
  - D. *Trichosporon beigeli*
5. A 24-year-old Vietnamese refugee was seen at a clinic in Houston. His chief complaints were weight loss and fever. A complete blood count confirmed he was suffering from anemia as well. Multiple skin lesions were present on his arms, some of them draining pus. Gram stain of the pus revealed what appeared to be yeastlike cells. A culture of the pus grew a green mould at 22°C, which produced a red soluble pigment (see Color Plate 33 ■). A lactophenol cotton blue wet mount of this organism is seen in Color Plate 34 ■. The causative agent in this case is
  - A. *Aspergillus fumigatus*
  - B. *Fusarium* sp.
  - C. *Trichoderma* sp.
  - D. *Penicillium marneffei*
6. A section of a lymph node stained with the Gomori silver and hematoxylin and eosin stains is shown in Color Plate 35 ■. A lactophenol cotton blue wet mount of a mould that grew from this specimen is shown in Color Plate 36 ■. Large, one-celled, smooth to tuberculate macroconidia and smooth or echinulate microconidia are typical of mycelial phase growth of
  - A. *Blastomyces dermatitidis*
  - B. *Coccidioides immitis*
  - C. *Histoplasma capsulatum*
  - D. *Paracoccidioides brasiliensis*
7. Which of the following types of *Candida albicans* infection is commonly acquired from an exogenous source?
  - A. Diaper rash
  - B. Neonatal thrush
  - C. Perianal infection
  - D. Urinary tract infection
8. In a direct examination of a KOH wet mount of a nail specimen, *Epidermophyton floccosum* could be detected as
  - A. Arthroconidia
  - B. Blastoconidia
  - C. Macroconidia
  - D. Microconidia
9. The mould phase of the systemic fungus *Blastomyces dermatitidis* can be confused with
  - A. *Scedosporium apiospermum*
  - B. *Sporothrix schenckii*
  - C. *Aspergillus* sp.
  - D. *Penicillium notatum*

10. It is usually difficult or impossible to identify a fungal culture before it is mature. However, hyaline, septate hyphae, and a young conidiophore with a foot cell (see Color Plate 37■) and a swollen vesicle are excellent clues to the identification of
  - A. *Acremonium*
  - B. *Aspergillus*
  - C. *Paecilomyces*
  - D. *Penicillium*
11. Zygomycetes are rapidly growing, airborne saprobes. In clinical specimens they
  - A. Are common as normal, human microflora
  - B. Are found only as contaminants
  - C. May be seen in a dimorphic tissue phase
  - D. May be found as a cause of rapidly fatal infection
12. *Trichophyton rubrum* and *T. mentagrophytes* may be differentiated by the
  - A. Consistently different appearance of their colonies
  - B. Endothrix hair infection produced by *T. rubrum*
  - C. Fluorescence of hairs infected with *T. rubrum*
  - D. *In vitro* hair penetration by *T. mentagrophytes*
13. Broad, coenocytic hyphae found in tissue would be most typical of infection with
  - A. *Aspergillus*
  - B. *Blastomyces*
  - C. *Microsporum*
  - D. *Rhizopus*
14. A fungus infecting only skin and nails typically produces in culture
  - A. Spindle-shaped, hyaline, echinulate macroconidia and microconidia
  - B. Cylindrical or club-shaped, smooth, thin-walled macroconidia and microconidia
  - C. Many microconidia in clusters or along the hyphae
  - D. Large, thin-walled, club-shaped macroconidia without microconidia
15. The most useful finding for prompt, presumptive identification of *C. albicans* is its
  - A. Failure to assimilate sucrose
  - B. “Feathering” on EMB
  - C. Production of chlamydospores
  - D. Production of germ tubes
16. Identify the dimorphic fungus that typically has a tissue phase in which the large mother cells have one to a dozen narrow-necked buds and a slowly growing mycelial form with intercalary chlamydospores and coiled hyphae.
  - A. *Blastomyces dermatitidis*
  - B. *Coccidioides immitis*
  - C. *Histoplasma capsulatum*
  - D. *Paracoccidioides brasiliensis*
17. Which of the following stains greatly enhances the visibility of fungi by binding to the cell walls, causing the fungi to fluoresce blue-white or apple green?
  - A. Rhodamine-auramine
  - B. Warthin-Starry
  - C. Calcofluor white
  - D. Periodic acid-Schiff



18. The formation of arthroconidia is *not* an important characteristic in the identification of
  - A. *Coccidioides*
  - B. *Geotrichum*
  - C. *Trichosporon*
  - D. *Sporothrix*
19. A black pigment produced by colonies growing on bird seed agar is due to
  - A. Urease
  - B. Phenol oxidase
  - C. Sucrose assimilation
  - D. Arthroconidia production
20. Which of the following fungi is *not* considered an opportunistic pathogen?
  - A. *Absidia*
  - B. *Aspergillus*
  - C. *Coccidioides*
  - D. *Fusarium*
21. Observation of hyaline or dematiaceous hyphae is an early clue in the identification of common, airborne fungi. Which of the following genera contains species found as dematiaceous contaminants?
  - A. *Alternaria*
  - B. *Aspergillus*
  - C. *Fusarium*
  - D. *Penicillium*
22. Which of the following fungi is most likely to be found as a common saprobe and as an agent of keratitis?
  - A. *Exophiala*
  - B. *Phialophora*
  - C. *Fusarium*
  - D. *Wamgiella*
23. The microscopic identification of *Pneumocystis jirovecii* is based on the detection of
  - A. Arthroconidia in subcutaneous tissue biopsies
  - B. Cysts and trophozoites in respiratory specimens
  - C. Yeasts in respiratory specimens
  - D. Tuberculate macroconidia in lung biopsies
24. Fungi that undergo asexual reproduction are termed
  - A. Imperfect
  - B. Perfect
  - C. Aseptate
  - D. Septate
25. Hyaline septate hyphae, branched or unbranched conidiophores, and multicelled banana-shaped conidia are characteristic of which of the following?
  - A. *Fusarium*
  - B. *Curvularia*
  - C. *Acremonium*
  - D. *Trichophyton*
26. Which of the following does *not* correctly describe the yeast *Rhodotorula rubra*?
  - A. It has been isolated from dairy products, soil, and water.
  - B. It is the most common fungal cause of diaper rash.
  - C. It has been identified as a nosocomial pathogen.
  - D. It has been found as a contaminant or commensal in specimens of urine, sputum, and feces.

27. A 21-year-old male member of a university track team presents to student health services with a light brown circular lesion on his upper back. The agent most likely responsible for this condition is
- Candida albicans*
  - Fusarium* spp.
  - Geotrichum candidum*
  - Malassezia furfur*
28. Which of the following is likely to be found in clinical specimens as normal microflora and as clinically significant isolates?
- Aspergillus niger*
  - Paracoccidioides brasiliensis*
  - Penicillium marneffei*
  - Candida albicans*
29. A 4-year-old child's hair is falling out in patches. The hair fluoresces when subjected to the UV light from a Wood's lamp. When the hair is cultured, a white cottony mould grows at 25°C on potato dextrose agar. Microscopically, rare microconidia, septate hyphae, and terminal chlamydospores are seen. Macroconidia are absent. The mould fails to grow on polished rice grains. The causative agent is
- Microsporum audouinii*
  - Microsporum gypseum*
  - Trichophyton mentagrophytes*
  - Trichophyton rubrum*
30. In tissues infected with *Histoplasma capsulatum*
- The hyphae usually invade blood vessels
  - Encapsulated yeast cells are typical
  - Tuberculate macroconidia are typical
  - The fungus is usually intracellular

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For each numbered mycosis below, choose the letter of the environment most commonly associated with an increased incidence of that infection.

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- Blastomycosis
  - Coccidioidomycosis
  - Cryptococcosis
- Lower Sonoran Life Zone
  - Mississippi and Ohio River basins
  - Pigeon roosts
  - Bat roosts

---

For each numbered mycosis below, choose the letter of the environment most commonly associated with an increased incidence of that infection.

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- Histoplasmosis
  - Sporotrichosis
- Sphagnum moss
  - Starling roosts
  - Stagnant fresh water
  - Colorado River Valley

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For each numbered incomplete statement, select the letter of the most appropriate species.

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- The cause of white piedra
  - The cause of black piedra
  - The cause of tinea nigra
- Hortaea werneckii*
  - Trichosporon* sp.
  - Piedraia hortae*
  - Fonsecaea compacta*

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For each numbered incomplete statement, select the letter of the most appropriate species.

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- 39. The cause of tinea versicolor
- 40. A keratinophilic saprophyte
- 41. A cause of otomycosis

- A. *Aspergillus niger*
- B. *Malassezia furfur*
- C. *Microsporum gypseum*
- D. *Geotrichum candida*

---

The incomplete statements below describe the appearance of growth of yeast or yeastlike fungi in morphology agar, such as rice agar or cornmeal agar with Tween 80, a finding helpful in the presumptive identification of these organisms. For each numbered description, select the letter of the most appropriate species.

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- 42. True hyphae and arthroconidia only
- 43. True hyphae, arthroconidia, and blastoconidia
- 44. Pseudohyphae, blastoconidia, and chlamydospores

- A. *Candida albicans*
- B. *Geotrichum*
- C. *Trichosporon*
- D. *Aspergillus fumigatus*

---

The incomplete statements below describe the appearance of growth of yeast or yeastlike fungi on morphology agar, such as rice agar or cornmeal agar with Tween 80, a finding helpful in the presumptive identification of these organisms. For each numbered description, select the letter of the most appropriate species.

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- 45. Pseudohyphae and blastospores only
- 46. Blastospores only, without hyphae or pseudohyphae

- A. *Mucor* sp.
- B. *Candida tropicalis*
- C. *Cryptococcus neoformans*
- D. *Candida albicans*

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Select the letter of the most appropriate specimen source for isolation of each numbered species description.

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- 47. *Cryptococcus neoformans*
- 48. *Histoplasma capsulatum*
- 49. *Pseudallescheria boydii*
- 50. *Trichophyton mentagrophytes*

- A. Bone marrow
- B. Cerebrospinal fluid
- C. Chronic draining sinus tract of foot
- D. Chronic interdigital lesion of foot

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# answers

# & rationales

1.

**C.** Areas of the San Joaquin Valley are highly endemic for *Coccidioides immitis*, and infectious arthroconidia of this fungus can be distributed in dust aerosols produced by construction and other disturbances. Symptomatic pulmonary disease patterns vary, but the signs and symptoms given are found in many cases. The fungus grows more rapidly than do other systemic fungal pathogens, and the aerial mycelium will typically produce the characteristic barrel-shaped arthrospores.

2.

**D.** The distribution of *Histoplasma capsulatum* is probably worldwide, but most clinical disease occurs in the western hemisphere. Most cases in the U.S. occur in the Ohio and Mississippi River valleys. This organism is found in areas contaminated by large amounts of bird excrement, such as starling and blackbird roosts. Inhalation of the spores results in a respiratory illness usually with

clinical symptoms within 2 weeks of exposure. Disease ranges from a mild influenzalike illness to acute fulminant lung infection resembling tuberculosis.

3.

**D.** *Sporothrix schenckii* is the agent of sporotrichosis. It usually enters the skin by traumatic implantation. This fungus grows *in vitro* as small yeasts at 35°C and as a mould at room temperature (22–30°C) with delicate hyphae and conidia.

4.

**C.** All species listed may be urease positive, but *C. terreus* does not grow at 35°C and may assimilate nitrate. *C. krusei* is inositol negative, and these species of *Candida* and *Trichosporon* produce hyphae on morphology agar. *C. neoformans* typically does not produce hyphae and is nitrate negative, is inositol and urease positive, and grows at 37°C.

5.

**D.** Infections due to *Penicillium marneffei* seem to originate in eastern and southeastern Asia. This fungus was first isolated in 1959 from a hepatic lesion from a bamboo rat, a rodent found throughout Southeast Asia. Clinical disease includes fever, weight loss, anemia, and death if untreated. Skin lesions may be present and may drain pus. Diagnosis is made via culture or histopathologic exam of lesions of skin, bone, or liver. The yeastlike cells of *P. marneffei* are oval (3–8  $\mu\text{m}$ ) and scattered throughout tissue. Elongated, sausage-shaped cells often contain cross-walls. At 22–30°C, structures typical of the genus *Penicillium* develop. At 35–37°C, round or oval yeastlike cells are seen.

6.

**C.** Diagnostic features of *H. capsulatum* include large, 8- to 14- $\mu\text{m}$  macroconidia with tuberculate projections. Tuberculate and smooth macroconidia may be seen in the same colony. Microconidia are also produced.

7.

**B.** Neonatal thrush is the oral candidiasis most commonly associated with mothers having vaginal *Candida*, and the newborn acquires the organism from the mother. Diaper rash due to *C. albicans* usually follows oral and perianal candidiasis of the infant. The other three infections are associated with physiologic changes in the host that permit proliferation of *C. albicans* already present in the host's microflora.

8.

**A.** KOH wet mounts should be used routinely for direct examination of nails, skin, or hair for fungal elements. KOH digests the keratinous tissue and facilitates observation of any fungi present. *Epidermophyton floccosum* and *Trichophyton* spp. invade nails, and the former typically is found as chains of arthroconidia in nail tissue.

9.

**A.** At 25–30°C, *Blastomyces dermatitidis* forms septate hyphae with delicate conidiophores of various lengths that bear round or oval conidia. It is important not to confuse the mould phase of *B. dermatitidis* with either *Scedosporium apiospermum* or *Chrysosporium* sp. *S. apiospermum* appears as septate hyphae with simple conidiophores of various lengths that bear oval conidia singly or in groups. *S. apiospermum* is the causative agent of mycetoma and can infect brain, bones, eyes, lungs, etc. *Chrysosporium* sp. appears as septate hyphae with simple to branched conidiophores that bear oval conidia. *Chrysosporium* sp. is commonly considered a contaminant.

10.

**B.** Conidiophores of *Aspergillus* arise from a foot cell and terminate in a vesicle. The vesicle produces phialides; the phialides then produce the conidia. Before the culture is mature, the presence of a young conidiophore with a foot cell and vesicle is a good clue to the identity of the fungus.

11.

**D.** Although generally found as laboratory contaminants, the zygomycetes can be clinically significant. Zygomycosis (mucormycosis) is an acute disease that often results in death within a few days in acidotic patients. Fungal agents of mucormycosis include *Rhizopus*, *Mucor*, and *Absidia*, which are common fungi found in the environment.

12.

**D.** When speciation of *T. mentagrophytes* or *T. rubrum* is not certain on morphology alone, the *in vitro* hair perforation test is useful; *T. mentagrophytes* is positive and *T. rubrum* is negative. Urease production by *T. mentagrophytes* is less reliable. Neither species produces endothrix infection, and *T. rubrum* rarely infects hair.

13.

**D.** *Rhizopus* and other fungal agents of mucormycosis are characterized by having coenocytic (nonseptate) hyphae. The finding of broad, nonseptate hyphal elements in sterile body fluids or tissue can provide rapid confirmation of a clinical diagnosis of mucormycosis. The other moulds listed have septate hyphae.

14.

**D.** *Epidermophyton floccosum* infects skin and nails. This dermatophyte produces thin-walled macroconidia, usually in clusters, but no microconidia. *Microsporum* spp. produce infections in hair and skin. *Trichophyton* spp. may produce infection of the nails, hair, and skin.

15.

**D.** Essentially all strains of *Candida albicans* produce germ tubes within 2 hours of incubation at 37°C in serum. Chlamydospores are produced by most strains of *C. albicans* after 24–48 hours at 22–26°C on cornmeal Tween 80 agar or a similar substrate. Use of eosin methylene blue medium to screen for *C. albicans* may require 24–48 hours of incubation.

16.

**D.** The dimorphic pathogenic fungi include the species listed. The parasitic or tissue phase of *P. brasiliensis* produces large, multiple-budding yeasts, 20–60 µm long. The saprophytic or mycelial phase colonies resemble *B. dermatitidis*, but all cultures produce intercalary chlamydospores and coiled hyphae, and conidia development is delayed or absent. Clinical types of paracoccidioidomycosis include relatively benign primary pulmonary infection; progressive pulmonary disease; disseminated disease; or an acute, fulminant, juvenile infection. The disease is endemic in certain areas of Central and South America.

17.

**C.** The calcofluor white stain requires the use of a fluorescence microscope. It is a rapidly staining method, requiring only one minute to complete. Stain binds to chitin in the cell wall of fungi.

18.

**D.** Barrel-shaped arthroconidia, alternating with empty cells, are typical of the mature mycelial phase of *Coccidioides immitis*. Species of *Geotrichum* produce chains of hyaline arthroconidia, and *Trichosporon* is characterized by production of hyaline arthroconidia, blastoconidia, hyphae, and pseudohyphae. *Aureobasidium* produces dematiaceous arthroconidia. Sporothrix is the sole member of the list that does not produce arthroconidia.

19.

**B.** Phenol oxidase breaks down the substrate found in niger seeds producing melanin. This result is characteristic of *C. neoformans*. *C. neoformans* is urease positive, but that reaction is not detected on this medium.

20.

**C.** *Absidia* and *Mucor* can cause the uncommon disease mucormycosis in debilitated patients. Rhinocerebral syndrome is the form of this infection most often seen in the U.S. Species of *Aspergillus* are ubiquitous and opportunistic and cause a variety of human infections. *Fusarium* is one of the saprobic fungi most often found in external mycotic keratitis following corneal trauma. *Coccidioides* is considered a true pathogen that can infect healthy people.



21.

**A.** Observation of dark pigmented hyphae in a culture is evidence that the fungus is in one of the dematiaceous genera. Typically, the reverse of a plate will be black. *Alternaria* is a common dematiaceous contaminant.

22.

**C.** Mycotic keratitis due to *Fusarium* has been reported following injury or cortisone treatment. An ulcerative lesion develops on the cornea. Corneal scrapings may be received for direct exam and culture.

23.

**B.** *Pneumocystis jirovecii* produces cysts and trophozoites that can be found in respiratory tract specimens. The fungus primarily infects the lungs, so specimens from the lower respiratory tract are most productive (e.g., bronchoalveolar lavage). Specimens can be stained with a silver stain or Giemsa stain.

24.

**A.** Fungi with only an asexual stage of reproduction are referred to as the imperfect fungi. Fungi able to reproduce sexually are called the perfect fungi. "Septate" and "aseptate" refer to the presence or absence (respectively) of cross-walls in hyphae.

25.

**A.** Diagnostic features of *Fusarium* spp. include hyaline septate hyphae and sickle- or banana-shaped macroconidia. Macroconidia are multi-septate with long or short branched or unbranched conidiophores. Microconidia (one or two celled) are also produced.

26.

**B.** *Rhodotorula rubra* has been isolated from soil, water, and a number of food sources, especially dairy products and as a contaminant of skin, lung, urine, or feces. *Rhodotorula* fungemia has been caused by contaminated catheters, intravenous solutions, and dialysis machines. *C. albicans* is a more common cause of diaper rash.

27.

**D.** *Malassezia furfur* is the causative agent of tinea or pityriasis versicolor—a superficial skin infection that occurs commonly on the upper back, chest, shoulders, upper arms, and abdomen. Initially lesions are discrete but in time may coalesce. Lesions may be hyper- or hypopigmented. *M. furfur* is part of the normal skin flora of over 90% of adults. There may be an association between the disease and excessive sweating. The disease is more common in tropical and subtropical areas.

28.

**D.** *C. albicans* is an endogenous species causing a variety of opportunistic infections. Infection is usually secondary to a predisposing debility. *Aspergillus* spp. are common saprophytic contaminants. *Paracoccidioides brasiliensis* and *Penicillium marneffeii* are dimorphic fungi that cause systemic mycoses.

29.

**A.** *Microsporum audouinii* most commonly affects children. Only rarely are adults infected. Colonies are flat, downy to silky, and gray to white in color. Colony reverse is salmon to brown with a reddish-brown center. Microscopic examination reveals septate hyphae, terminal chlamydoconidia, and occasional microconidia (borne singly). Macroconidia are very rare or absent. Infected hair fluoresces. Growth on polished rice grains aids in differentiating *M. audouinii* from other *Microsporum* species that grow well on rice grains.



30.

**D.** *Histoplasma capsulatum* is found primarily within histiocytes and in macrophages or monocytes in specimens from bone marrow aspirates, biopsies, or the buffy coat of centrifuged blood. Unstained cell wall of the tissue (yeast) form of *H. capsulatum* may be mistaken for a capsular halo in stained preparations. Only the mould phase would exhibit hyphae and macroconidia.

31.

**B.** *Blastomyces dermatitidis* is rarely found in the environment, and there is no reliable skin test for screening for past or subclinical blastomycosis. Outbreaks occur most frequently following exposures to moist environments like streams and rivers. The incidence of clinical cases in the U.S. is highest in the Mississippi and Ohio River basins and part of the Missouri River drainage.

32.

**A.** The most highly endemic regions of coccidioidomycosis are semiarid, with dry, hot seasons and wetter, cooler seasons above freezing. The areas of the southwestern U.S. and northern Mexico with this typical Lower Sonoran Life Zone climate have the highest incidence of coccidioidomycosis. The peak endemic period is fall, when the fungus becomes airborne from the desert surface.

33.

**C.** Although *Cryptococcus neoformans* does not appear to infect pigeons, it apparently passes unharmed through their gut. It has been found in large numbers, even as the predominant microorganism, from the debris of old pigeon roosts. Viable, virulent, desiccated cells, small enough to be inhaled into the alveoli, can be present in the dust of these roosts.

34.

**B.** The most highly endemic areas of histoplasmosis (Missouri, Kentucky, southern Illinois, Indiana, and Ohio) also have the most starlings, whose flocks produce large accumulations of guano. *Histoplasma capsulatum* has been found growing in almost pure culture in accumulated starling guano. Exposure to aerosols containing many spores of this fungus has been associated with a number of "common source" outbreaks of histoplasmosis.

35.

**A.** In temperate countries, including the U.S., sporotrichosis is an occupational hazard of gardeners and nursery workers and is frequently associated with contact with sphagnum moss. In Mexico, it has been associated with working with grass, and a well-known epidemic in South Africa involved gold mine workers in contact with untreated mine poles. *Sporothrix schenckii* produces subcutaneous infections that begin at the site of traumatic implantation.

36.

**B.** White piedra is frequently caused by *Trichosporon ovoides* and *T. inkin*. *T. beigeli* was the name formerly used for the species infecting humans. The disease is characterized by soft, white to light brown nodules around and in the hair shaft. The nodules are composed of hyphae, yeastlike arthroconidia, and sometimes blastoconidia. The beard and body hair are more often affected than scalp hair.

37.

**C.** Black piedra is caused by *Piedra hortae*, which produces brown to black, gritty nodules on the outside and under the cuticle of the hair shaft. Scalp hair is the site most often involved. Direct microscopic examination of portions of these nodules in KOH wet mounts can show septate dematiaceous hyphae and ascospores.

38.

**A.** *Tinea nigra* is a superficial skin infection caused by *Hortaea werneckii*. The pigmented, painless lesion, which usually occurs on the palms or fingers, may be mistaken for melanoma. Accurate laboratory findings in a KOH preparation of a skin scraping are important in preventing surgical mutilation of the patient. Microscopic examination of skin scrapings from tinea nigra shows dematiaceous, septate hyphae and budding cells.

39.

**B.** *Tinea versicolor* is a chronic, mild, superficial skin infection caused by *Malassezia furfur*, which may also be found on normal skin. Despite the name “tinea versicolor,” the causative fungus is not a dermatophyte. Skin scrapings from the lesions demonstrate characteristic yeastlike cells and hyphae.

40.

**C.** *Microsporum gypseum* is a keratinophilic fungus (dermatophyte). It has a geophilic species that has been isolated from human infections. It is a moderately rapid grower, producing numerous thick-walled rough macroconidia.

41.

**A.** *Aspergillus niger* causes approximately 90% of the otomycoses and external ear infections due to fungi. *Aspergillus fumigatus* also causes otomycosis. Other fungi, far less often involved, include *Scopulariopsis*, *Penicillium*, *Rhizomucor*, *Candida*, and other species of *Aspergillus*.

42.

**B.** *Geotrichum* spp. typically produce numerous hyphae and arthroconidia. Germinating arthroconidia of *Geotrichum*, however, may be mistaken

for blastoconidia production. This may cause confusion between *Geotrichum* and *Trichosporon*.

43.

**C.** *Trichosporon* spp. produce hyphae and arthroconidia. They may also produce blastoconidia, although these may be rare. If present, blastoconidia can differentiate *Trichosporon* from *Geotrichum*.

44.

**A.** *Candida albicans* and *C. dubliniensis* both produce pseudohyphae and are germ tube positive. Both are capable of producing chlamydospores and blastoconidia. These two species are difficult to differentiate.

45.

**B.** *Candida tropicalis* typically produces long-branched pseudohyphae. Blastoconidia are produced singly or in short chains. This species does not produce chlamydospores. The carbon assimilation pattern of *C. tropicalis* resembles that of *C. albicans*, and some strains of *C. tropicalis* may produce a positive germ tube test if incubated more than 3 hours.

46.

**C.** *Cryptococcus neoformans* produces only blastoconidia when growing on morphology agar (e.g., cornmeal agar with Tween 80). This species is usually identified by its encapsulated cells, production of urease, failure to assimilate nitrate, and production of brown pigment on bird seed agar. Cryptococcosis can lead to systemic infections in immunocompromised patients.

47.

**B.** The most frequently diagnosed form of cryptococcosis is central nervous system infection. Few or many organisms may be in the cerebrospinal fluid, but a clinical diagnosis of meningitis can often be confirmed by the cryptococcal antigen test. In the past, the use of a microscopic examination of a spun specimen with India ink has been used. The cryptococcal antigen test is much more sensitive and is the recommended test.

48.

**A.** *Histoplasma capsulatum* is a parasite of the reticuloendothelial system and is seldom extracellular. Specimens such as sternal bone marrow, lymph node, liver and spleen biopsies, or buffy coat of blood should be stained with Giemsa or Wright's stain and examined for small, intracellular yeast cells.

49.

**C.** *Pseudoallescheria boydii* is the most common cause of eumycotic mycetoma in the U.S. Mycetoma is a clinical syndrome of localized abscesses, granulomas, and draining sinuses that develops over months or years. It usually occurs on the foot or hand after traumatic implantation of soil organisms.

50.

**D.** *Trichophyton mentagrophytes* is a common cause of intertriginous tinea pedis or athlete's foot. This is a chronic dermatitis most often affecting the areas between the fourth and fifth and third and fourth toes. The acute inflammation often subsides, but recurrences are common.

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